Methods for determining nutrient requirements in pregnancy—I[1]

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Nutrient requirements are determined by genetic heritage and the somatic response to environmental factors. The potentials for growth, reproduction, resistance to disease, intellectual development, and longevity are provided by genetic heritage. Biochemical and physiological responses to environmental stimuli are conditioned by experience and nutritional status. Nutrient requirements may vary with alterations in physiological status and, because of genetic and somatic imprinting, may differ quantitatively among individuals. In provision for individual variations, nutrient requirements should be studied in groups, and averages as well as extremes of requirements determined.

Several approaches have been used to estimate nutrient requirements. The most indirect approach is the study of experimental animals. Definition of nutrient requirements of animals under different physiological conditions, such as pregnancy, rapid growth, infections, trauma, and severe stress has provided valuable insights. Interactions among nutrients, and between nutrients and nonnutrients have been defined. Some of these findings have application to humans.

Findings from animal studies that may have implications for human nutritional requirements in pregnancy are illustrated in **Figure 1**. Nitrogen utilization depended on the intakes of both zinc and nitrogen of rats who were fed a moderately zinc-deficient diet (6 ppm) and studied on day 20 of gestation (1). Together, zinc and nitrogen intakes accounted for 93% of the variance in nitrogen retention. The amounts of zinc and nitrogen required for a given level of nitrogen retention were related to each other. If either nutrient was inadequate, nitrogen retention was impaired. From this experiment, we are reminded of the fundamental principle that nutrients act in concert with one another to facilitate metabolic processes, and if they are not available in appropriate mixtures, metabolism is impaired.

A second, though insensitive, method for determining human nutrient requirements is the nutrition survey (2). The distribution of nutrient intakes within a population is estimated and related to clinical and laboratory findings. Persons who do not show clinical or laboratory evidence of deficiency are presumed to have had an adequate nutrient intake. This approach has several weaknesses. Perhaps most serious is the difficulty of quantitative measurements of dietary intakes. Accurate histories require alert, communicative respondents who are aware of their diets, and skilled interviewers who have a knowledge of local foods, methods of preparation, and customs. Conversion of history data to quantitative measures of nutrient intake requires analysis of the foods eaten by the respondents. Presently available food composition tables usually provide average figures from several laboratories and regions. Contents of most individual food items are within 10% of the calculated values for energy and protein but not for many other nutrients. In addition, factors that affect the bioavailability of the nutrients are usually not considered in food tables. Thus, findings from dietary histories often do not correlate well with clinical signs of nutritional status, with the possible exception of signs associated with severe deficiencies.

The weakness of dietary histories for estimation of nutrient requirements of individuals is illustrated by the finding that histories were not good predictors of the requirement for energy (3). Energy required by 11 volunteers to maintain body weight for 90 days, or

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The American Journal of Clinical Nutrition 34 APRIL 1981, pp. 697–704. Printed in U.S.A.

longer, under controlled conditions was calculated from food composition tables. Energy utilization was determined by bomb calorimetry of representative diets and excreta. Mean values were within 10% of the calculated values. The energy expenditures of the volunteers from exercise while in the metabolic units, were similar to their levels before entering the unit. However, detailed admission dietary histories resulted in a predicted energy requirement that was 20 to 30% in error. In contrast, the formulas of Harris and Benedict (4), Kleiber (5), and the Mayo Foundation (6) were less than 10% in error when the calculated basal energy requirement was increased by 50% for energy expended beyond basal conditions. Finer individualization of energy expended over basal only slightly improved the prediction (**Fig. 2**).

The clinical spectrum of nutritional status

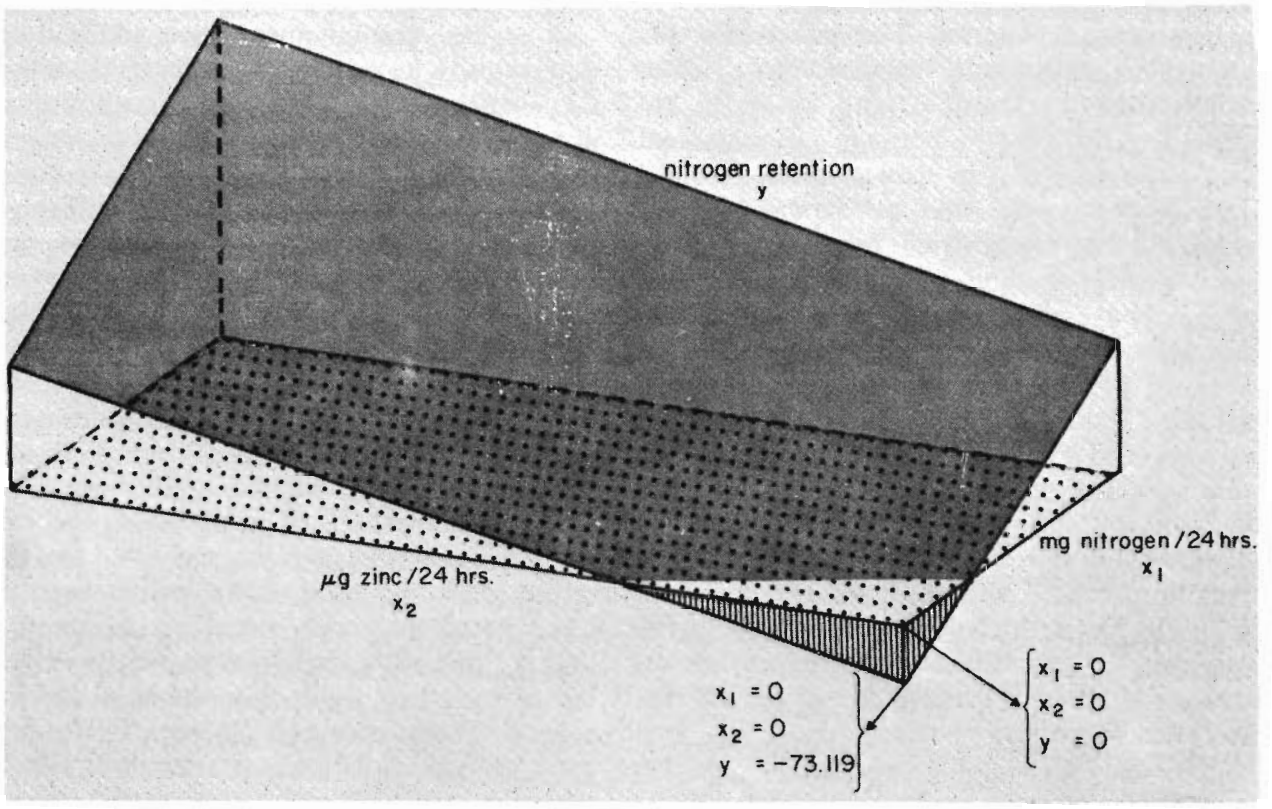

FIG. 1. Representation of the relation of nitrogen and zinc intake to nitrogen retention in pregnant rats (1). This computer simulation was based on the equation: $y = -73.119 + (0.412)x_2 + (0.201)x$; $p < 0.0001$, $r^2 = 0.93$.

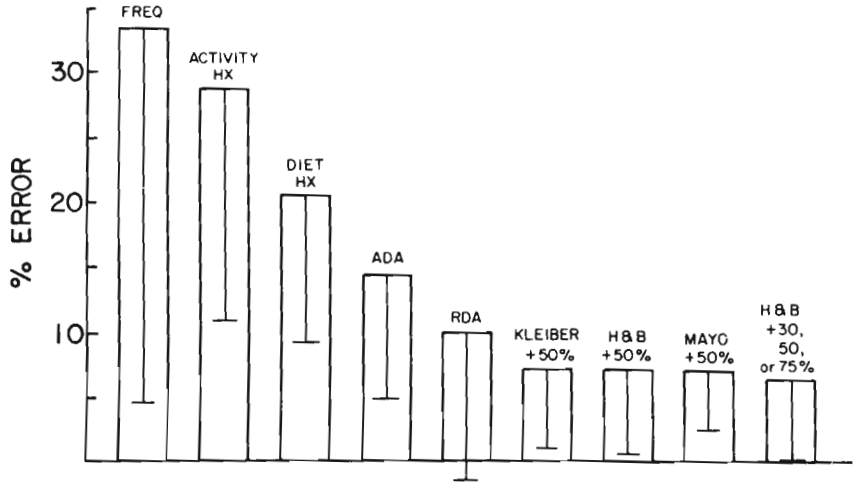

FIG. 2. Representations of the percentage error (mean ± SD) in predicted energy requirements from actual requirements (3). Freq, food frequency questionnaire; Hx, history; ADA, American Diabetes Association/American Dietetic Association Guide; RDA, NAS/NRC Recommended Dietary Allowance; Kleiber, Kleiber formula; H & B, Harris and Benedict formula; Mayo, Mayo Foundation nomogram.

(**Fig. 3**) is a second impediment to the use of the survey method for estimating requirements. At one extreme is severe malnutrition, the clinical signs of which are obvious, although not always specific. A far greater proportion of a population with malnutrition exhibits marginal or mild depletions for which clinical signs are much less specific. Separations between marginal and mild malnutrition on the one hand and adequate nutrition on the other are indistinct. Sensitivity of the indexes used for diagnosis may shift an individual from one category to the other. Laboratory indexes of nutritional status are usually more sensitive than clinical signs (**Table 1**).

Physiological changes, such as hemodilution, can markedly change plasma levels of some nutrients independent of nutritional status. **Figure 4** shows the distribution of plasma zinc levels in women at wk 20 of pregnancy contrasted to levels of plasma zinc in nonpregnant control women in the study discussed by Dr. Metcoff elsewhere in this workshop. **Figure 5** shows the trend downward of plasma zinc throughout pregnancy observed by Jameson (7). Jameson interpreted his data as showing evidence of zinc deficiency in mothers in his population. The deficiency was reflected by a strong association between decreased levels of plasma zinc and complications of labor, as well as malformations in infants. Dr. Metcoff and I have been unable to confirm his findings. Perhaps differences between the two study populations account for our failure to confirm. In any case, it seems evident that specific laboratory indexes may, under some circumstances, correlate with physiological abnormalities, and therefore be interpreted as subnormal; under other circumstances, similar levels of the index may not correlate with physiological abnormalities. In the latter instance it is more difficult to conclude that the low level of the index is a sign of abnormality.

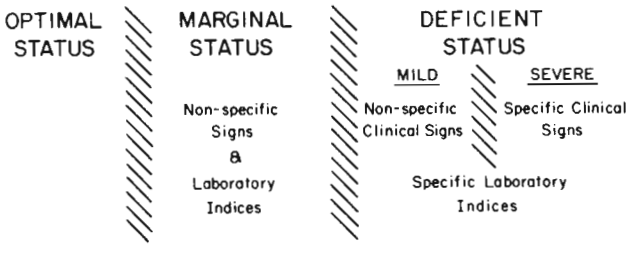

FIG. 3. Spectrum of nutritional status and its relation to homeostasis and environment.

TABLE 1
Some indices of nutrient status

Nutrients	Test
Thiamin	Erythrocyte transketolase, serum thiamin, urinary thiamin, neurological function.
Riboflavin	Erythrocyte glutathione reductase, serum riboflavin, urinary riboflavin.
Pyridoxin	Serum pyridoxal phosphate, erythrocyte pyruvic transaminase, urinary xanthurenic acid excretion after tryptophane load, erythrocyte indices, neurological function.
Vitamin B_{12}	Urinary methylmalonate excretion, serum vitamin B_{12}, erythrocyte indices, leukocyte morphology, neurological function.
Folic acid	Serum folate, red blood cell folate, urinary FIGLU after histidine load, erythrocyte indices, leukocyte morphology.
Ascorbic acid	Serum vitamin C, leukocyte vitamin C.
Vitamin A	Serum vitamin A, dark adaptation.
Vitamin D	Serum 25 and 1-25 $(OH)_2$ vitamin D.
Protein	Serum albumin, hair root morphology, plasma RNase.
Niacin	Urinary N-methylnicotinamide, neurological function.
Iron	Serum ferritin, erythrocyte protoporphyrin, serum iron, serum transferrin, saturation of iron binding protein, erythrocyte indices, hemoglobin.
Zinc	Plasma/serum zinc, erythrocyte zinc, urinary zinc, salivary zinc, hair zinc, taste and smell acuity, serum alkaline phosphatase, dark adaptation.
Copper	Serum/plasma copper, ceruloplasmin, hair copper.
Selenium	Plasma selenium, erythrocyte uptake of ^{75}Se, erythrocyte glutathione peroxidase.

For some nutrients, several indexes of deficiency are available (Table 1). Sensitivity of the index can be crucial to the detection of a deficiency and, hence, identification of the frequency of a deficiency in a population. Iron deficiency is a case in point. With iron depletion, serum ferritin, which reflects body iron stores, is depressed (**Fig. 6**), but, other

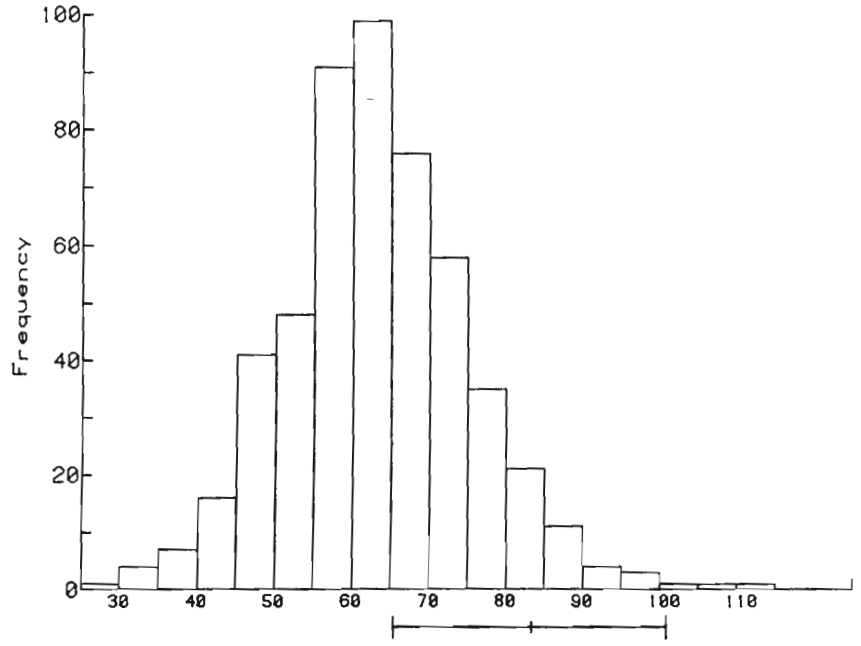

FIG. 4. Distribution of plasma zinc (μg/dl) of women at about 20 wk of pregnancy compared to 35 nonpregnant control women (mean ± 2 SD).

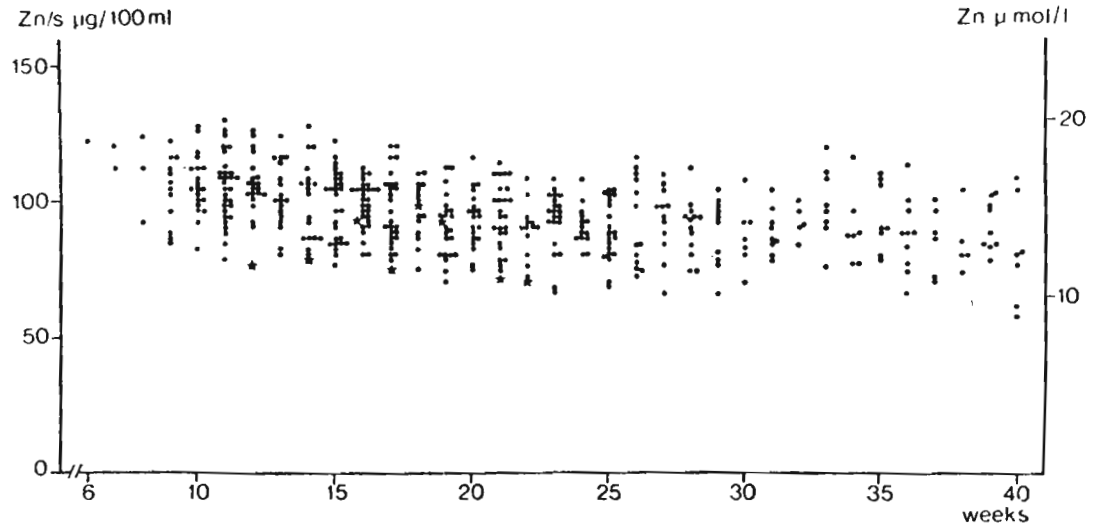

FIG. 5. Plasma zinc levels of women throughout pregnancy (7). *Points* indicated by *stars* represent women whose infants had malformations.

indexes of iron status are unchanged. If indices less sensitive than serum ferritin had been used to ascertain iron status in this man, no evidence of depletion would have been detected, the diet would have been presumed adequate, and the iron requirement for maintenance of iron stores would have been underestimated. Similar errors may be made in population studies if sensitive indices are not used.

The increase in plasma copper and ceruloplasmin during pregnancy is an example of an alteration, caused by estrogen in an index (8), that might obscure assessment of nutrient status. If dietary intakes of copper by pregnant women are marginal, they might not be reflected by the plasma levels, although copper content of liver is decreased and certain metabolic functions altered. Such depletion of liver copper, without a decrement in serum copper, has been observed in nonpregnant rats given large doses of histidine (9), an amino acid that is excreted in large amounts during pregnancy (10). Thus, under some circumstances, plasma levels of a nutrient may be poor indicators of nutritional status, and indexes that reflect metabolic function might be preferable.

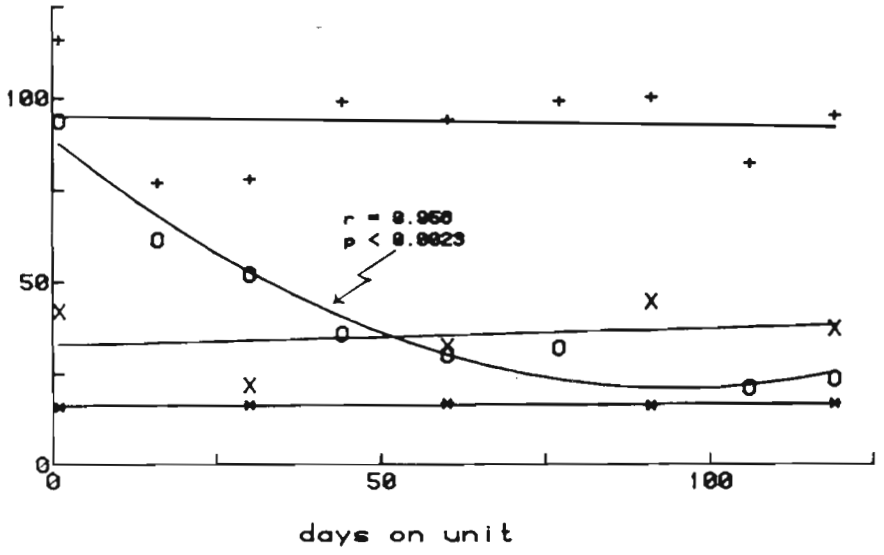

FIG. 6. Serum ferritin change of an adult man fed a diet containing about 15 mg of iron daily, in response to monthly phlebotomy of about 200 ml. On the y axis x = percentage saturation, + = μg of iron/dl, o = ng of ferritin/ml, and # = g of hemoglobin/dl.

Despite these problems, surveys can provide useful estimates of dietary levels of nutrients above which deficiencies are unlikely. The estimates can be improved in some instances by use of therapeutic trials in which, after an initial survey, specific nutrients believed deficient are administered individually. Dietary histories of the nonresponders and responders to treatment provide a rough estimate of the levels of nutrients that are sufficient to meet requirements. This approach may be unsuccessful, however, in populations with multiple simultaneous deficiencies. In such populations, treatment with a single deficient nutrient may result in no response, or may be followed by signs of deficiency of a second limiting nutrient. Thus, a clear therapeutic response to a specific nutrient may not occur until the associated deficiencies are cured (11). This complication in therapeutic trials can be avoided by supplementing all subjects with nutrients that are not under investigation, and treating only a portion of the population with the test nutrient, thus, providing a control and experimental group.

A third method for determining nutrient requirements is the factorial method. Data required for the calculation include body content and daily losses of the nutrient. From this information, the amount of the nutrient that must be absorbed for maintenance of the body content and for growth can be calculated. **Figure 7** shows the results of a factorial

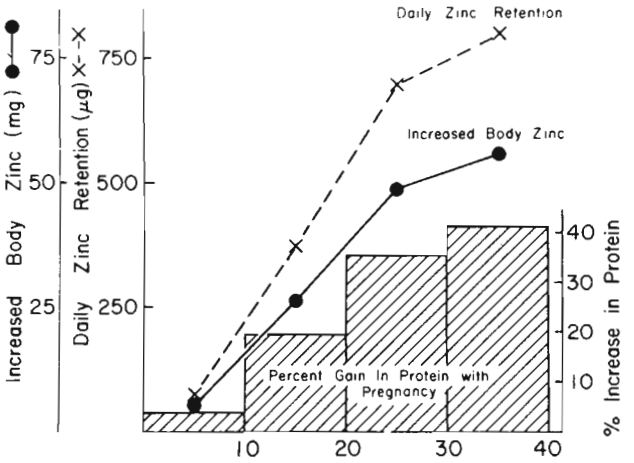

FIG. 7. Factorial estimate of increased zinc requirement of pregnant women in 10-wk intervals (12).

estimate of the zinc requirement of pregnant women (12). The principle weaknesses of this approach are limited knowledge of body composition, and an incomplete understanding of absorption, retention, and loss of nutrients from the body. Despite these limitations, the method has been used to calculate provisional dietary requirements for zinc (13) (**Table 2**). These "provisional requirements" serve as a "best guess" for clinical and public health use until more definitive data become available.

Requirements can be estimated with more assurance than the above methods provide, if subjects are studied prospectively in a controlled environment. However, even under these conditions, methods are far from perfect. The most commonly used method is the

TABLE 2
Factorial basis of provisional requirements of dietary zinc in relation to estimates of assumed losses and availability (13)*

Age	Peak daily retention	Urinary excretion	Sweat excretion	Total required	Milligrams necessary in daily diet if content of available zinc is:		
					10%	20%	40%
	mg	*mg*	*mg*	*mg*			
Infants							
0–4 mo	0.35	0.4	0.5	1.25	12.5	6.3	3.1
5–12 mo	0.2	0.4	0.5	1.1	11.0	5.5	2.8
Males							
1–10 yr	0.2	0.4	1.0	1.6	16.0	8.0	4.0
10–17 yr	0.8	0.5	1.5	2.8	28.0	14.0	7.0
18 yr plus	0.2	0.5	1.5	2.2	22.0	11.0	5.5
Females							
1–9 yr	0.15	0.4	1.0	1.55	15.5	7.8	3.9
10–13 yr	0.65	0.5	1.5	2.65	26.5	13.3	6.6
14–16 yr	0.2	0.5	1.5	2.2	22.0	11.0	5.5
17 yr plus	0.2	0.5	1.5	2.2	22.0	11.0	5.5
Pregnant women							
0–20 wk	0.55	0.5	1.5	2.55	25.5	12.8	6.4
20–30 wk	0.9	0.5	1.5	2.9	29.0	14.5	7.3
30–40 wk	1.0	0.5	1.5	3.0	30.0	15.0	7.5
Lactating women	3.45	0.5	1.5	5.45	54.5	27.3	13.7

* The above estimates are based on the assumption that the fat-free tissue concentration of zinc in man is approximately 30 μg/g (14). This figure is equivalent to 2.0 g of zinc in the soft tissues of an adult male and 1.2 g in the soft tissues of an adult female. The zinc requirements at various ages was determined from the change in lean body mass with age (15). Bone zinc was not included in these calculations, as zinc in bone is relatively sequestered from the metabolically active pool of body zinc. The excretion of zinc in sweat is based on an assumed zinc content in sweat of 1 mg/l (16). The estimated requirement for lactation is based on a zinc content in milk of 5 mg/l (17) and a daily milk secretion of 650 ml. The urinary excretions of zinc are based on levels reported (18, 19).

"balance technique". By this method, it is theoretically possible to determine the level of nutrient intake that is necessary for maintenance of equilibrium. It is also possible to evaluate interactions among nutrients, and between nutrients and nonnutrients.

The limitations of the balance technique have been reviewed (20). One of the most serious is the time necessary for homeostatic and gastrointestinal equilibration, following changes in diets. Fecal markers have been used to measure the time of clearance of a meal from the gastrointestinal tract. A recent report, contrasting experience with polyethylene glycol and chromic oxide, suggests that the two markers do not traverse the intestine at the same rate, and that neither is consistently quantitatively recovered (21). Thus, markers are not entirely satisfactory. An alternative to markers is a long period of equilibration before starting collections. Prolonged collection periods are used to minimize the effects of daily fluctuations in fecal flow. If possible, subjects who defecate at least daily are chosen. The time necessary for equilibration of any individual can be determined only by serial observations. Studies with fecal markers suggest that the intestine is cleared in most individuals by 18 days after a meal (21). As far as homeostatic equilibration is concerned, the time required varies widely among nutrients.

Another unresolved problem with balance studies is the apparent, continued accumulation of some nutrients, such as nitrogen and minerals, when they are fed at levels above the requirement. While it seems obvious that homeostatic mechanisms must prevent indefinite continued accumulation, documentation of homeostatic adjustments is incomplete. Because of these and other problems (20) balance studies should always be interpreted with caution.

There are two basic approaches to use of

the balance technique for determining requirements. In the first approach, subjects are fed a constant diet containing a marginal level of the test nutrient. The test nutrient is then added to the diet in a step-wise manner until the balance becomes positive. This approach may be unsatisfactory because of homeostatic mechanisms. In addition, the order of feeding may influence the level of supplementation at which equilibrium is achieved. In the second approach, groups of subjects are fed constant diets containing the test nutrient at levels of intake that are determined by individual energy needs, and the balance of each subject is estimated. Theoretically, the point of equilibrium will be determined by regression analysis of the balance data against the intake. If the levels of the nutrients in the diets fed the subject are such that the balance values fall about equally on both sides of zero (**Fig. 8**) one's confidence in the apparent requirement is enhanced; greater caution in interpretation may be advisable however if the data fall predominantly on one side of the zero line (**Fig. 9**). Confidence in the findings is enhanced when the correlation coefficient is high.

A fifth method, for assessing requirements, depends on measurement of specific metabolic or physiological responses, or the levels of nutrients and their metabolites in plasma and urine. This approach has been particularly useful in the study of vitamin requirements. In one application of this method,

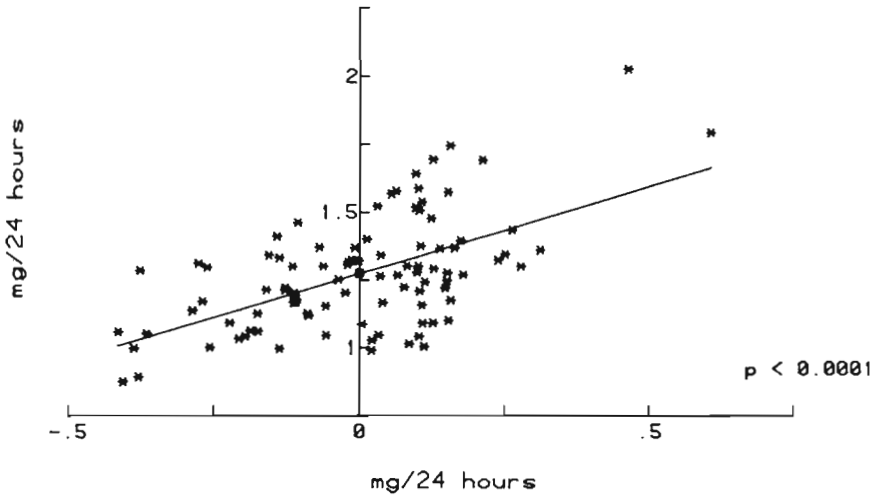

FIG. 8. Regression analysis of copper balance versus intake to determine dietary copper requirement of men fed 100 to 135 g of protein daily, N = 100, r = 0.5828, p < 0.0001.

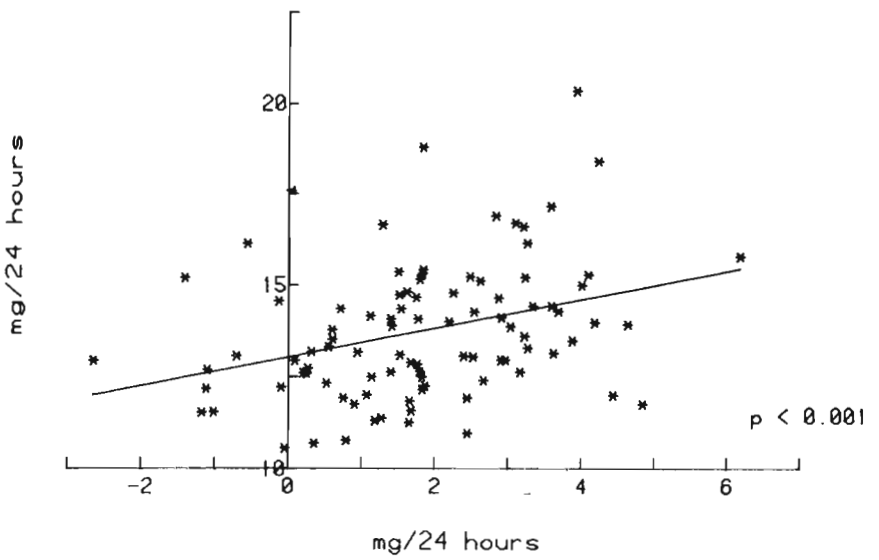

FIG. 9. Regression analysis of zinc balance versus intake to determine dietary zinc requirement of men fed 100 to 135 g of protein daily, N = 94, r = 0.3315, p < 0.001.

subjects are fed a constant diet that is deficient in the nutrient under study. Biochemical and physiological indices are monitored closely, and after abnormalities become apparent, the nutrient is given in small, increasing doses, until the index of deficiency returns to normal. The level of intake necessary to restore and maintain normality is presumed to be the requirement. This method for determining requirements has obvious limitations for studies during pregnancy.

A second approach is to define the metabolic indexes that might reflect nutritional abnormalities and then supplement with the nutrient in question to determine the amount of nutrient needed to prevent the abnormal metabolic phenomena. The increased xanthurenic acid excretion observed in pregnancy is such an index. Studies of the effect of vitamin B_6 supplementation on xanthurenic aciduria and measurements of plasma pyridoxal phosphate have suggested to some observers that vitamin B_6 requirements are increased in pregnancy (22).

Summary

There are several methods for evaluating nutritional requirements. Under appropriate circumstances, all can provide useful information. The most precise methods are those in which subjects live under controlled conditions. None of the methods is foolproof. The cost increases with the degree of control. Therefore, less expensive, less precise methods, may be appropriate for preliminary studies. If preliminary findings are positive, more carefully controlled studies should be done to obtain definitive information.

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